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Prevalence of ecto- and endoparasites of the Sea bass *Dicentrachus Labrax* in Port Said Governorate, Egypt.

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ABSTRACT

The Sea bass (*Dicentrachus labrax*) is consider one of the most important seawater fish species of South Europe and Mediterranean aquaculture. In the present study; we investigated the parasitism of Sea bass in Port Said Governorate, Egypt during the period from October 2016 till October 2017. A total number of 100 sea bass (ranged from 300 to 400 gm weight) were collected randomly; the parasitological examination showed a total of 9 different parasite species were found. The ectoparasites were represented by two copepods (*Caligus elongates* and *Lernanthropus psciaenae*) with 28% total prevalence, one isopod species (*Levonica redmani*) infestation rate was 16%, and one monogenean species; *Diplectanum aequans* which showed the highest infection rate (64%). Five species of digenean were found with a total prevalence of 36%; they were (*Acanthostomum spiniceps*, *Erilepturus Hamati*, *Stephanostomum murielae*, *Creptotrema creptotrema*, and *Aponurus mulli*). The highest total prevalence of infestation among the examined fish was in autumn (60%) and the lowest was in summer (24%), while in winter and spring the prevalence rates were 48% and 36% respectively.

INTRODUCTION

The Sea bass (*Dicentrachus labrax*) consider one of the most important seawater fish species of South Europe and Mediterranean aquaculture (**Scapigliiati *et al*., 2002**). Besides, The Sea bass is the first non-salmonid species to be cultivated commercially in Europe and even the most important commercially cultured fish species grown in sea cages in the Mediterranean (**Banu and Zafer, 2012**). Egypt produces about19, 027 tons from European sea bass (**GAFRD, 2014**). Fish parasites may cause great losses either by injuries and even death to the fishes which act as a final or intermediate hosts for them (**Shih and Jeng, 2002**). Parasite remains one of the most important pathogens categories, many species of parasites affecting marine fishes and they cause high economic losses in the marine culture sector in Egypt. Also, they can be found in various tissues and organs of fish (**Abou Zaid *et al.*, 2018**), they can retard the fish growth, leading to diseases and may even cause the deaths of the fish (**Ahmet, 2013**). For instance, Trematodes may infest the fish as an adult, juvenile worms or encysted metacercaria, and they classified to digenetic trematodes and monogeneans. The digenetic trematodes are very common and represent the largest group of all internal parasites **(Eissa *et al*., 2017**). Monogeneans are one of the most important helminthes of the external surfaces of the fish, the attachment and the feeding of monogenean induce histopathological changes to the epithelium, leading to the invasion of a wide range of secondary fungal, bacterial and viral infections (**Yardimci and Pekmezci, 2012**). In addition to the crustacean parasites which are one of the serious problems of aquaculture with their morbidity and mortality beside, they have been reported in cultured fish in the Mediterranean region (**Noor El-Deen *et al*., 2013**). There are About 2,000 species of parasitic arthropods have been reported and most of them are belonging to the class Copepoda, particularly genera, *Lepeophtheirus*, *Caligus*, and *Pseudocaligus* which have effects on their hosts, consequently, they can cause high mortalities (**Watchariya and Nontawith, 2008**). The isopods such as *Livoneca redmani* infect the gills of fish bilateral or unilateral infection led to hyperplasia of lamellar epithelium and congested blood vessels, so caused economic loss associated with reducing the fish growth and drop of fish production in lake Qurun (**Helal *et al*., 2018**). Besides that **Elghayaty and Tadros (2019)** revealed that isopods affect the quality of fishes so become unacceptable from consumers which leads to economic loss. The aim of the present study to investigate the parasitism of sea bass in Port Said Governorate, Egypt, for one year. The morphological identification of the isolated parasites and both total and seasonal prevalence of parasites infested examined Sea bass were recorded.

MATERIALS AND METHODS

Sample collection and clinical examination:

A total number of 100 sea bass (ranged from 300 to 400 gm weight) were collected randomly from fishing port at Port Said governorate, Egypt, during the period of one year from October 2016 till October 2017. The fish samples were transferred to the laboratory in fiberglass tanks partially filled with marine water and supply with aerator according to **Noor El-Deen *et al.* (2013).**

Parasitological examination:

Macroscopic examination

The samples were examined grossly for any abnormalities on whole body surfaces, external gross lesions, and for the presence of ectoparasites**.**

Microscopic examination

Skin and fins were scraped with a scalpel blade whereas, the gills were dissected out and separated in Petri dishes and examined microscopically for any detached parasites. The detached monogeneans or copepods and isopods were removed utilizing stereomicroscopy (**Abou Zaid *et al.*, 2018**).

Smear preparations, permanent slides

Monogeneans were isolated and mounted, copepods and isopods were fixed in 70% alcohol (**Bunkley - Williams *et al.*, 2006)** and identified based on their morphological features. The digeneans were washed in saline, fixed in cold AFA (alcohol-formalin-acetic acid) (**Georgi and Georgi, 1992)**, stained with alum carmine, dehydrated and then cleaned in xylene and mounted in Canada balsam (**Abou Zaid *et al.*, 2018**). All parasites were identified using selected identification keys of **Yamaguti (1958, 1961, and 1971).**

Ethics:

This study was conducted following legal ethical guidelines of the Medical Ethical Committee of the National Research Center, Dokki, Egypt.

RESULTS

1: Prevalence of parasitic infection among examined *D.labrax:*

The analysis of 100 samples of sea bass revealed high parasite diversity. A total of 9 different parasite species were found. The ectoparasites were represented by two copepods (*Caligus elongates* and *Lernanthropus psciaenae*) with (28%) total prevalence, one isopod species (*Levonica redmani*) infestation rate was 16%, and one monogenean species which was *Diplectanum aequans* which showed the highest infection rate (64%) and five species of digenea were found with total prevalence 36%, they were (*Acanthostomum spiniceps, Erilepturus Hamati, Stephanostomum murielae, Creptotrema creptotrema,* and *Aponurus mulli*) **(Fig. 1).**

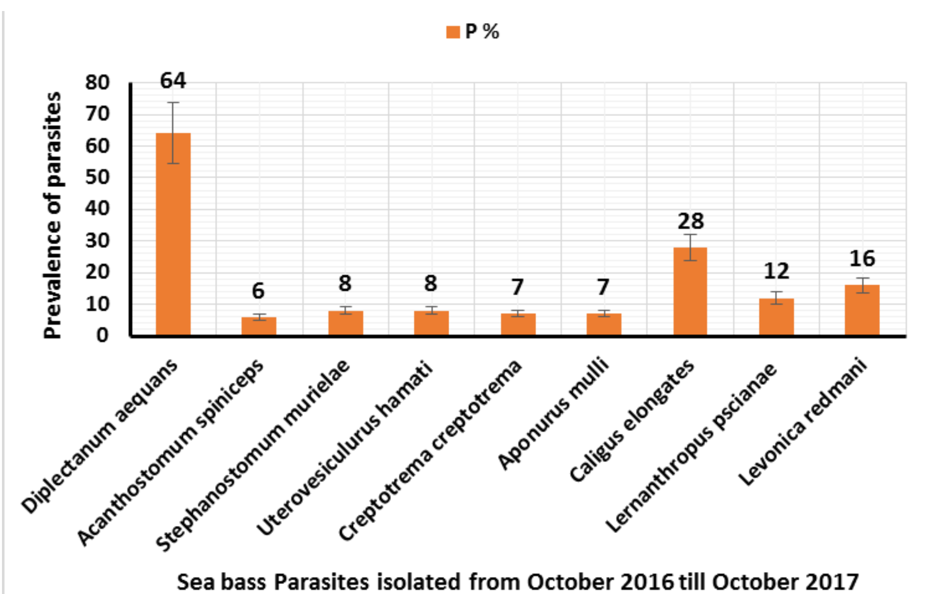

Fig. 1: Parasites of the sea bass (*Dicentrarchus labrax*) collected from Port Said Governorate - Egypt, during the period of one year from October 2016 till October 2017.

2: Seasonal prevalence of the parasites infestation among the examined fishes:

The present results revealed that the highest total prevalence of infestation among the examined fish was in autumn (60%) and the lowest was in summer (24%), while in winter and spring the prevalence rates were 48% and 36% respectively, **(Fig. 2).**

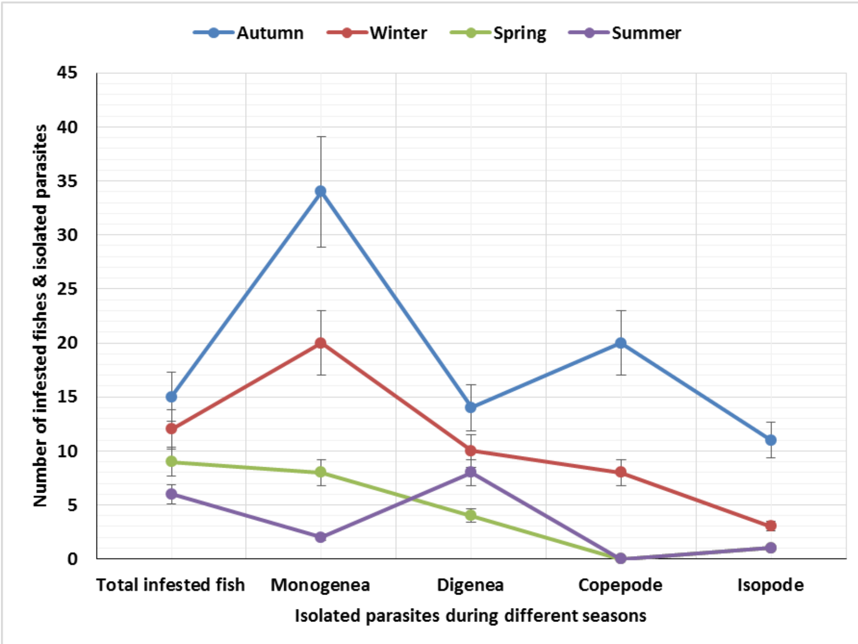

Fig. 2: Seasonal frequencies of the isolated parasites from the examined *Dicentrarchus labrax*.

3- Morphological description of the collected parasites:

3.1- Morphological description of the collected copepods

A- *Caligus elongates* Nordmann, 1832

Crustacean parasite copepods were collected from the gills of infested fish. The body length of the female measures 3.2 mm, the male parasite is 2.1 mm. the body is flattened, elongated with brown spotted coloration, and has two characterized long bar-shaped egg pouches or strings **(Fig. 8c).**

B- *Lernanthropus psciaenae* Blainville, 1822

The female was cylindrical and measured 3.2 mm in length and 0.9mm in width at the middle of the body. The head is separated by a constriction from the rest of the body. The dorsal plate is situated posterior, the first thoracic leg is biramous, and the exposed of the first segment bear blunt distal spines. A tiny papilla-like process is located at the base of the endopod. The 5th legs consist of a single lamella. The egg strings are elongated and have 127-149 uniseriate, strongly flattened eggs; meanwhile, the male body is slender and measured 1.8 mm in length and 0.6mm in width. The mandible is slender and has 7 denticles. The 1st maxilla consists of 3 segments; the terminal is conical. The 2nd maxilla consists of 3 segments; the terminal segment is provided with 2rows of blunt teeth and spine, **(Fig. 8a, 8b).**

2.2- Morphological description of the collected isopod species:

Levonica redmani Leach, 1818

The body is ovate and mostly twisted to one side, light brown in color with dark chromatophores. Cephalon is not projecting between the bases of the antennae. Its Posterior margin appears trilobed .one pair of eyes are located laterally. 2 pairs of antennae appear, the last two appear narrower pereopods are robust and characterized by large dactyli. Pleon: It is somewhat narrower than pereon and not immersed in it. It is of 6 segments that decrease gradually in width toward the posterior **(Fig. 3, 4).**

3.3-Morphological description of the isolated trematode parasites:

1- Gill monogeneans:

Diplectanum aequans Wagener, 1857

Family : Diplectanidae

A small monogenetic trematode with a maximum length1.3 mm. the prohaptor has 4 cephalic lobes with two groups of head glands and two pairs of eye spots. The opisthaptor has a squamodisc, two pairs of large anchor, and 14 marginal hooks, **(Fig. 6b).**

2-Gastrointestinal digeneans:

Examination of mucosal scraping of the gastrointestinal tract revealed:

a-*Acanthostomum spiniceps* Looss, 1896

Family: Cryptogonimidae

Body elongated, slightly truncated posterior end. Oral sucker terminal, funnel-shaped, Outer surface of oral sucker provided with a row of 23 large, simple peri-buccal spines. Pharynx large, ovoid, and strongly muscular. Esophagus relatively short and wide, Intestinal ceca well developed, opening at the posterior end via anal pores. Testes tandem or slightly diagonal, located near the posterior end of the body being of irregular rounded shape. Ovary is submedian or partially median, pretesticular. Uterus coiled from side to side in intercecal field between ovary and acetaulum, eggs small, very numerous. Vitellaria extends from behind seminal vesicale to preoverian level, **(Fig. 5a).**

b- ***Uterovesiculurus hamati (Erilepturus Hamati)* Yamaguti, 1934**

Family: Hemiuridae

Body elongate, stout, fusiform. The oesophagus is short and broad. Testes two, post acetabular, round, symmetrical, and nearly equal in size. Genital pore is at the posterior ventral margin of the pharynx. The ovary is post-testicular, globular, slightly median. Receptaculum seminis well developed, situated close behind the ovary. Uterus much coiled and muscular and metraterm opens in to the hermaphroditic duct. Eggs small and numerous, **(Fig. 5b, 5c).**

c- ***Stephanostomum murielae*** Looss, 1899

Family: Acanthocolpidae

Body elongate, narrow, widest in the region of ventral sucker or testes. Tegument spinous. Oral sucker is terminal. Ventral sucker rounded. Forebody is relatively long. Prepharynx long. Pharynx elongate oval. Oesophagus short. Intestinal bifurcation in posterior forebody. Caeca long and narrow, Testes 2, oval, entire, tandem, contiguous. Post–testicular region short. Cirrus–sac relative short. Ovary oval to round, entire, contiguous, or very close to the anterior testis, **(Fig. 6a, 6c).**

d- ***Creptotrema creptotrema*** Travassos *et al.,* 1928

Family: Allocreadiidae

Body small, oval to elongate, subterminal oral sucker with horses shoe-shaped flap, pharynx short, Testes in the posterior third of the body, irregularly oval, symmetrically to slightly oblique. The ovary is posterolateral to the acetabulum, mostly on the dextral side of the body. **(Fig. 6d).**

e-***Aponurus mulli*** Looss, 1907

Family: Lecithasteridae

Body elongated, Tegument smooth. Oral sucker subterminal, spherical. Ventral sucker large,

muscular, spherical, located between the first and second parts of the body. Pre-pharynx absent. Pharynx muscular, sub-globular, overlapped posterior margins. Caeca terminated close to the posterior extremity, ends obscured by uterine loops, difficult to observe.

Testes were two in number, sub-globular, contiguous, very close to the ventral sucker. Genital pore a wide median slit at the level of mid-pharynx. Small sinus-organ observed protruding

through the genital pore. Uterus extensive in hind body, main bulk dorsal to gonads, posterior to vitellarium. Vitellarium well-developed, ventral, and slightly overlapping posterior to the ovary **(Fig. 7).**

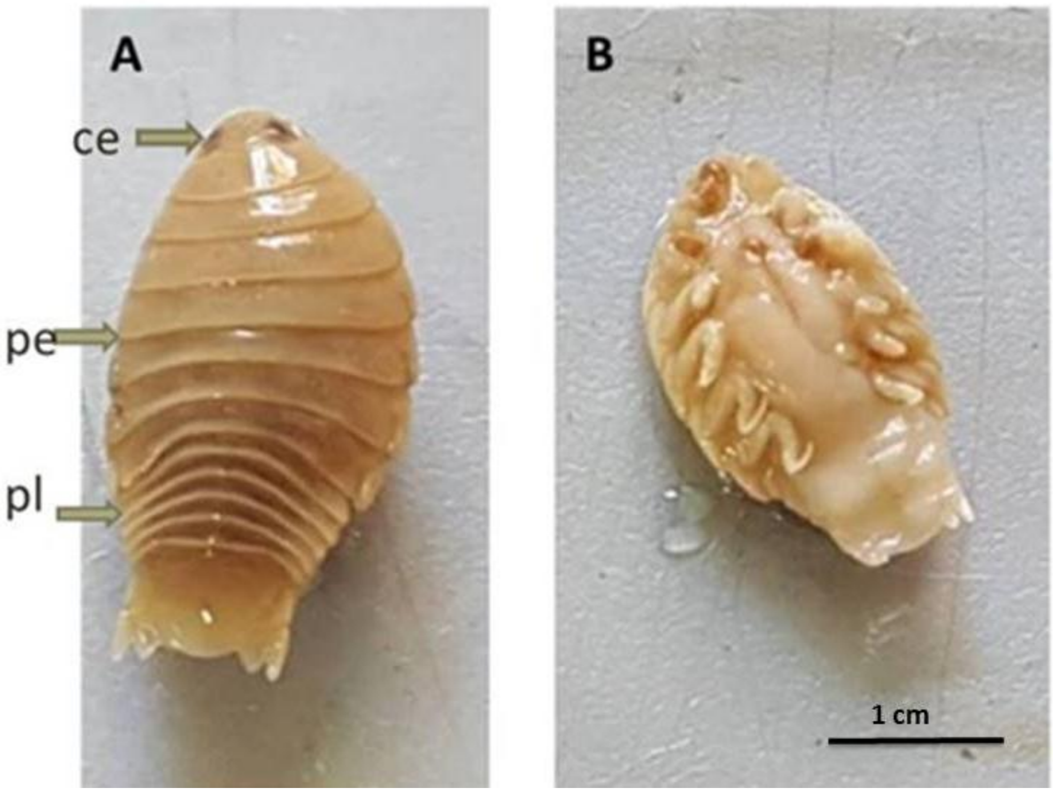

Fig. 3: (A) dorsal view ***Levonica redmani***; **(B):** ventral view ***Levonica redmani***
ce: cephalon, **pe.**: pereon, **pl:** pleon

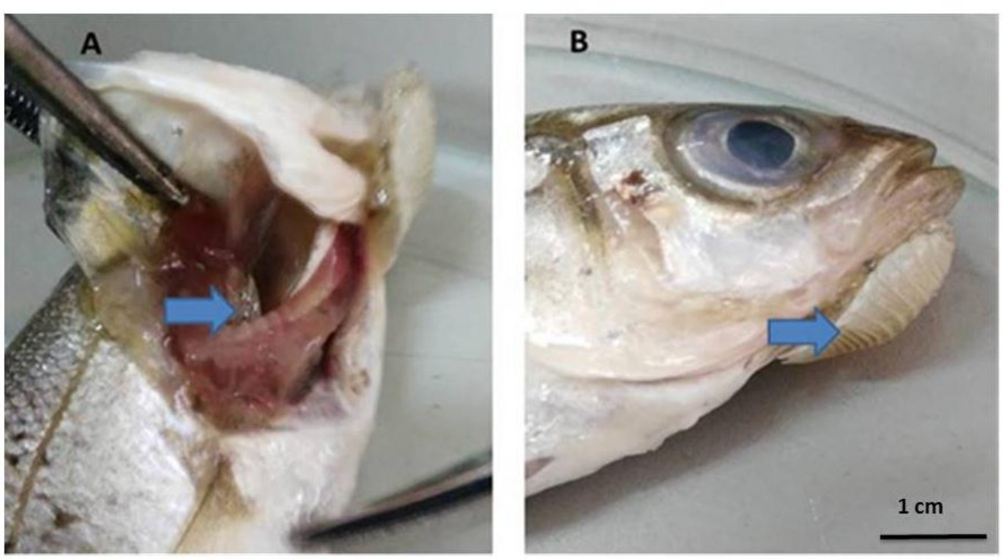

Fig.4: the sites of ***Levonica redmani*** attaching (**A**) gill attachment (**B**) External attachment

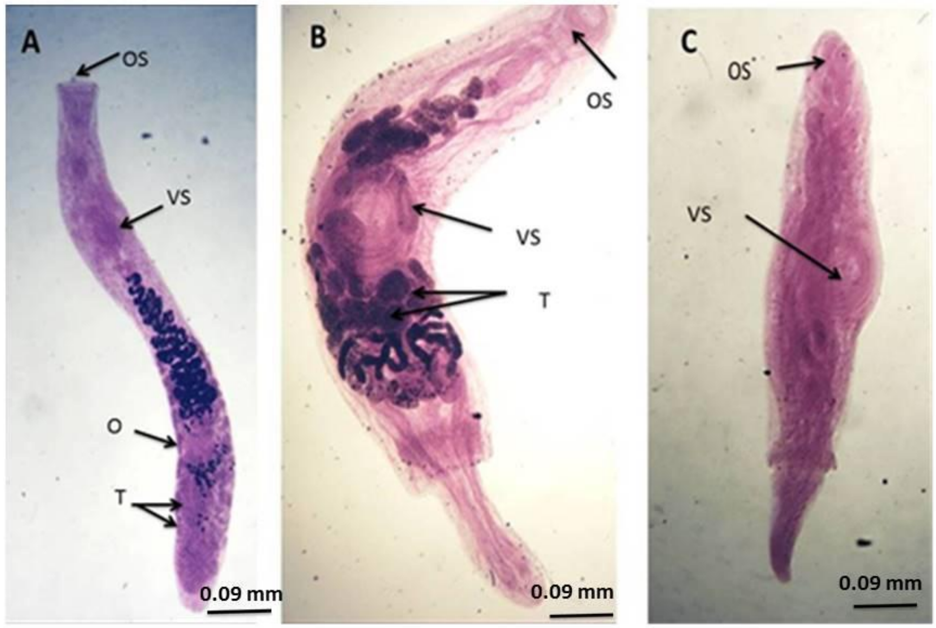

Fig.5: (A) ***Acanthostomum spiniceps*; (B)& (C): *Uterovesiculurus hamati*, (*Erilepturus hamati)* O**: Ovary, **O.S.**: Oral Sucker, **T:** Testis, **V.S.:** Ventral sucker

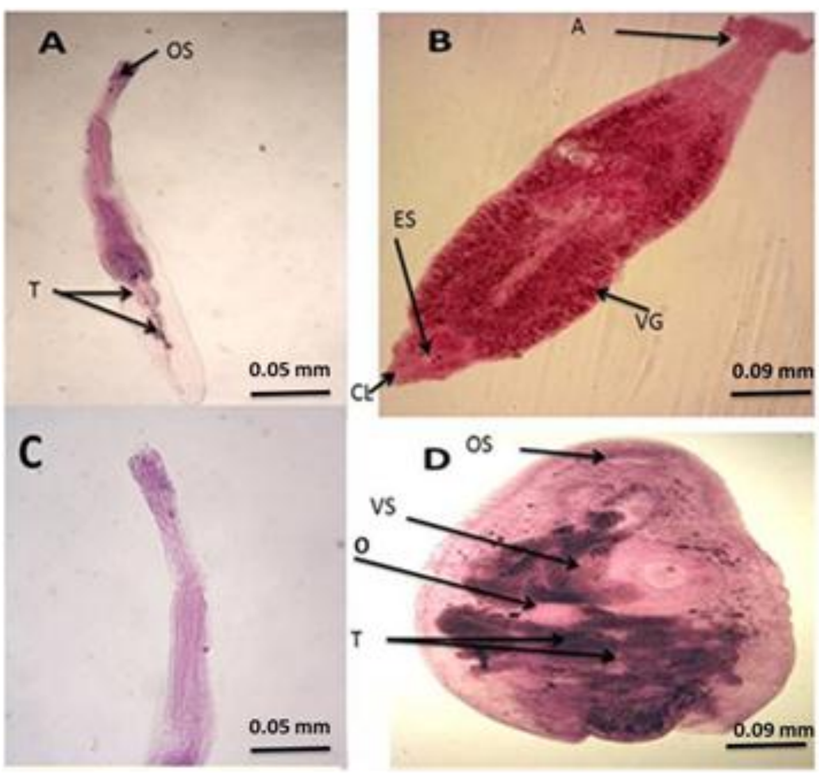

Fig.6:(A)*Stephanostomum murielae*; (B): *Diplectanum aequans* (C): *Stephanostomum murielae* ant. Part; (D): *Creptotrema creptotrema*
O: Ovary, **O.S.**: Oral Sucker, **T:**Testis, **V.S.:**Ventral sucker, **A**: anchor**, VG**: vitelline gland, **ES**: eye spots, **cl**: cephalic lobe.

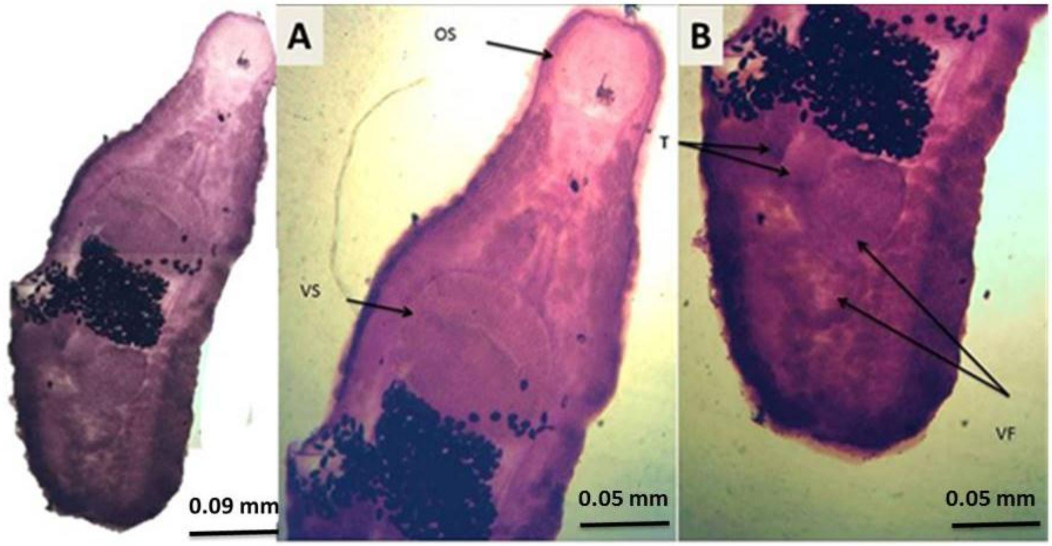

Fig.7: ***Aponurus mulli*** **(A)**: anterior part showing **O.S.**: Oral Sucker, **V.S.:**Ventral sucker, **O**: Ovary (**B**)**:** posterior part showing **T:**Testis, **V.F.:** Vitellarium

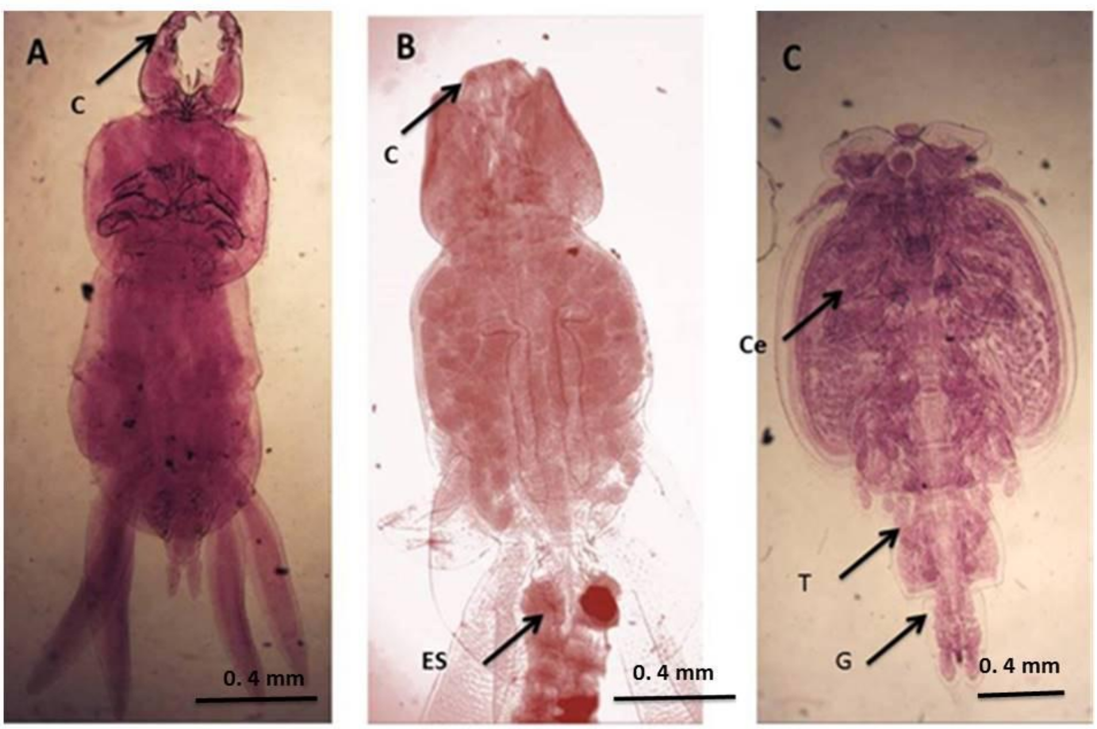

Fig.8: Crustacean copepode (**A**)**: (*Lernanthropus pscianae*)** Whole male copepode **(B): (*Lernanthropus pscianae*)** Whole female**; (C):** (***Caligus elongates)***
C:Claws, **E.S.**:egg sacs, **Ce**:Cephalothorax, **T**: Thoracic seg., **G:**genital complex

DISCUSSION

Fish parasitic diseases are causing great economic fish production losses especially in tropical countries like Egypt (**Ahmet, 2013**). In the present study, the analysis of the samples of sea bass revealed high parasitic diversity and a total of nine different parasite species were found. Regarding the total prevalence of infestation, the higher infestation rate was recorded with the monogenetic trematodes (*D. aequans*) (64%) and these results were in agreement with **Mohamod and Razak (2011)** who detected that the prevalence of *D. aequans* was 66.6%, and less than **Yardimci and Pekmezc (2012)** who recorded a high prevalence rate of *D. aequans* infestation in cultured sea bass which was 100%. The prevalence of digenetic trematodes was (36%) which was nearly similar to the results obtained by **Youssef and Derwa (2005**) who reported that the prevalence was (28.4%) from *Sardinella sp.* and *Morone labrax* (24%) **(El-Lamie, 2007)**. While it was less than that obtained by **(El-Ashram and Shager, 2008)** (61.3%) and more than **Raef (1990)** from marine fish (16.47%) and **Abd el-Mawla and Abo-Esa (2011)** from siganus fish (4.6%). The prevalence rates of copepod and isopod were 28% and 16% respectively, This result was nearly in agreement with the result recorded **by EL-Lamie (2007)**, it was 15.67% and **El-Raziky (2016)** which was 18%. On the other hand, the result was lower than the results obtained with **El Boghdady (2015)** which was 41.9% and **Faisal (2008)** which was 60%, from a different fish species *Mugil cephalus*, and **Eissa *et al.* (2017)** which was 58.5% from *Dicentrarchus labrax* and *D.punctatu*, but it was higher than the result recorded by **El-Razky (2009)** which was 7%. The difference between the prevalence rates may be due to the difference in fish species and the localities from which fish was obtained **(Eissa *et al.*, 2017).**

Seasonally, the prevalence of the collected monogenea was the highest in autumn (53%), followed by winter (31%), while spring and summer were 13% and 3% respectively, this result agreed with **Rawson and Rogers (1972)** who found monogenean parasites showed peaks of abundance during cold seasons and disagree with **El Lamai (2007)** who recorded the highest prevalence of monogenea in sea bass was in spring.

The seasonal prevalence of digenean trematode infestation also was highest in autumn and winter 39% and 28% respectively, followed by summer 22% and spring 11%. These results were nearly agreed with that of **El-Lamai (2007)** who recorded the highest infestation in winter, while **Eissa *et al.* (2017)** recorded the highest infestation was in spring.

Moreover, the seasonal prevalence of crustacean infestation has the maximum values in autumn (copepods, 71%) and (isopods, 69%) and winter (copepods, 29%) and (isopods, 19%), while their minimum in spring and summer. This result was in agreement with **Abu Samak and Ashraf (2008)** who found that the infestation rates with the same parasite reached their maximum values (42.5% and 35%) in autumn and winter respectively and with **Banu *et al.* (2014)** who noticed a gradual increase in the prevalence of such parasite fall to winter after examination of specimens of the European Sea bass

Dicentrarchus labrax which were infested with *Caligus minimus*, this result is in disagreement with that obtained by **Eissa *et al.* (2017)** who recorded the highest infestation of crustacean was found in spring 84%, followed by summer 80% and with **Noor El-Deen *et al*. (2013)** who recorded high prevalence of infested marine seabass and mullet in summer season was 91.6 and 71.3% respectively, followed by spring season was 51% and 90% respectively and absent in autumn and winter seasons. These changes in the prevalence may be related to the differences of the location from which the fishes were collected (**Eissa *et al.*, 2017)** and the changes in the immune response of fish at different temperatures **(Kennedy and Walker, 1969),** or it may be related to changes in plankton and food composition e of *D. labrax* in different seasons. **(Tekin *et al.*, 2008).**

Regarding the parasitological examinations, one monogenetic trematode was isolated from the gills (*Diplectanum aequans)* which is also detected by **Abou Zaid (2018)** from sea bass; *Dicentrarchus labrax.* In addition to six digenean trematodes that were isolated from the intestine, the 1st digenean trematode was detected is *Acanthostomum* sp. This result agreed with **Eissa *et al*. (2017*)*** who isolated *Acanthostomum* from Cultured Marine Fishes and also agreed with **Abdel-Gaber *et al.* (2018).** Regarding the 2nd one is *Creptotrema creptotrema* which is also recorded by **Kohn (1984)** and **Arredondo (2013),** the 3rd is *Stephanostomum murielae* which were listed by **Bray and Cribb (2008)** and the 4th trematode species is *Aponurus mulli* which was previously detected by **Carreras-Aubets *et al.* (2011)** and **Abdel-Gaber *et al.* (2018),** the last digenean trematode is *Uterovesiculurus hamate* its morphological description was agreed with that given by **Ratnakala *et al.* (2013)** who collected Erilepturus hamati from the intestine of *Lates calcarifer* beside **Willis *et al.* (1966)** who detected the species of Erilepturus from *Alectis indica, Rastralliger kanagurta*, and *Muraenesox cinereus*.

The crustacean copepods collected were *Lernanthropus pscianae* and *Caligus elongates*, this morphological description was agreed with that given by **Noor El-Deen *et al.* (2013)** and **Abdel-Mawla *et al.*** (**2012**) who isolated the same parasites from *Morone labrax.* The only isopods collected were *Levonica redmani* which is in agreement with that detected by **Mahmoud *et al.* (2017).**

CONCLUSION

The present study revealed high parasite diversity which has dangerous impacts consequently solutions should be found to decrease parasitic infestations especially in winter and autumn.

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